

On *Thismia Aseroe* (Beccari) and its Mycorrhiza.

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With Plates XIII and XIV.



A. MORPHOLOGY.

A NUMBER of holosaprophytic forms found in the tropics of the old and new worlds, constitute the Burmanniaceous genus *Thismia*. The species here described, *T. Aseroe*, is Malayan, and was discovered and first described by Beccari¹.

The plant consists of a system of branching, cylindrical, leafless, axial structures, which will be hereafter alluded to under the name of absorbing organs. These organs extend horizontally over a substratum of decaying leaves to which they are attached². When the plant is in the flowering condition, an erect scale-bearing inflorescence-axis rises up from the creeping absorbing organ. This axis was tentatively described by Beccari as simple, and terminating in a solitary flower; but frequently it is branched. In my specimens a second flower was always visible in the axil of

¹ Beccari, Malesia.

² Mr. H. N. Ridley informs me that the plant lives in damp shady spots of the forest, and that the habit and habitat are as above described.

one of the three scales forming an involucre beneath the terminal one: but there may be as many as three flowers in the inflorescence (Fig. 19). As is often the case with saprophytes devoid of chlorophyll, the general body of the plant is not white: in this instance it is pink.

Morphology of the Flower. (Fig. 20.)

The flower is orange-yellow in colour. The perianth forms a trumpet-like tube with six linear segments radiating from its summit like rays of a star. The six stamens are opposite the perianth-segments; they are inserted on the perianth-tube near the top, and are partially shaded by the thickened rim of the tube. The stamens hang down, and their connectives are dilated and, continuing below the anthers, cohere to form a tube. In fact the stamens are fused except at their points of insertion and at the apices of the connectives. Thus a tube is formed lying within the perianth-tube and open at both ends. This staminal tube is perforated at the top by six window-like narrow openings which are the gaps between the separately inserted stamens. The structure of the stamens is complicated and requires full description. Their structure on the face towards the centre of the flower is simple; they form a tube (with the above gaps) which has a plain surface merely rendered papillose by numerous blunt short hairs. This condition persists right down to the median free tooth which terminates the connective below. Looking through the tube from the inside not far below their point of attachment, the six anthers may be seen, and lower down, on radii alternating with the anthers, are six discoid opaque bodies—the nectaries. These last were seen and figured by Griffiths in *T. Brunonis*, but their nature was not understood by him. Both the anthers and the nectaries open towards the exterior, not towards the centre of the tube. Hence the anthers are really introrse though they open away from the centre of the flower. The structure of the stamens on their outer faces is complex.

Above is the short membranous filament which is attached both above and at its sides to the top of the tube of the perianth. So the filaments form six little dome-like chambers which stand over the six anthers. A transverse section taken through this region would show that there are two wings to the filament which already denote the method of formation of the staminal tube. Lower down, these wings meet and form a tube, and the pollen-sacs opening outwards (towards the perianth-tube) are visible. Below the anthers the fused wings continue, and form six furrows between the thickened ridges which are continued down from the anthers. In the middle of the furrows are long hairs which, I imagine, serve to catch the pollen. Descending lower the ridges become winged, and the two wings of the adjacent ridges tend to arch over the furrows. Below this the wings increase in size, and form six small tunnels in which lie the six nectaries. Each discoid nectary consists of a single layer of palisade-like, deeply-staining cells. Half of the disk belongs to one stamen and half to an adjacent one, and the line of fusion of the two stamens is recognizable, as is the distinct curvature of the nectariferous cells (Fig. 18). At the upper end of the tunnel the wings have long hairs which help to roof in the cavities in which the nectaries lie. Lower down the nectaries are replaced by hairs in the middle of the furrows, and the staminal ridges become much changed. They form curved plate-like outgrowths, attached above and at the sides, so that six little flattened dome-like chambers are formed closed above but open below. The median free part of this curved plate-like outgrowth hangs down and is rolled slightly inward and has conspicuous long hairs at its margins. Continuing downwards, only the lateral walls of this open chamber persist. The staminal tube therefore exhibits at this point twelve little channels, the broader ones representing inter-staminal radii, and the narrow ones the staminal radii. Below the connectives separate, and the lateral walls already mentioned end in conspicuous strong teeth or pegs. The median portion of the connective persists lower and tapers to a fine

tooth. The ovary is inferior, unilocular with three parietal placentae. The ovules have long filaments and develop very late. The style surmounted by three-segmented stigma is short, reaching up to the point of the terminal teeth of the connectives. The flower is proterandrous. It will be seen that both the pollen and nectaries are very completely hidden and protected. The structure of the flower suggests that it is cross-pollinated by means of small flies. I imagine that they crawl down the staminal tube, go on to the stigma as a landing place at which to turn round in order to crawl up to the nectaries. It seems probable that they then walk up the nectar-tunnels, and being unable to turn round continue up and emerge through one of the six windows in the top of the staminal tube, accidentally taking, en route, some pollen either from the pollen-sacs or from the hairs on the furrows.

It is usually stated that the perianth-tube and the top of the ovary fall off together when the fruit is ripe. Such is not the case. The perianth wholly severs its connexion with the ovary before the fruit ripens, and subsequently the lid of the ovary separates as an operculum. (See Fig. 19.)

Histology of the Inflorescence-axis.

The inflorescence-axis at its base bears a few very small scale-leaves crowded together; higher up the scales increase in size and are separated by distinct internodes till close under the terminal flower there is an involucre of three relatively large scales.

The axis itself is not terete, but is more or less winged.

At the growing-point a *dermatogen* is visible, but in mature parts there is no definite regular epidermis preserved; a number of the cells have peeled off. The superficial cells have only a very delicate cuticle on their outer walls. They are narrow and elongated longitudinally. Fungal hyphae run over the surface but do not penetrate.

The cortex is composed of about ten layers of parenchymatous cells with not very small intercellular spaces. Amongst these cells are some raphide-mucilage sacs. At the base

where the axis is inserted on the absorbing organ, straight hyphae run longitudinally in the cortex and are continuous with the mycorhizal hyphae; they stretch only a short distance up the inflorescence-axis.

The *endodermal* cells are uneven in size: they have thin suberised walls; the lateral walls have the dots (which however are not invariably on walls actually radial).

The *stele* is considerably larger than in the absorbing organ. There is a single somewhat irregular layer representing the pericycle. Within are three collateral bundles and a parenchymatous pith.

The *phloëm* is tangentially extended, and consists of sieve-tubes with terminal plates, companion-cells, and less protoplasmic parenchymatous cells.

The *xylem* is better developed than in the absorbing organ. The protoxylem, represented by narrow disintegrating spiral and annular vessels, is not exactly radially internal to, but slightly on the side of the mass of xylem. Besides these vessels there are in each bundle 4-8 (in transverse section) broader intact spiral and annular tracheae, the segments of which are long and tapering.

Histology of the Scales.

The scales are simple in structure. An epidermis with thin cuticle and devoid of stomata covers a mesophyll consisting of two layers of large parenchymatous cells including small intercellular spaces. A single vascular bundle with xylem and phloëm runs up the middle of the scale. The only point of interest in the leaves is that they contain amongst the mesophyll-cells many raphide-mucilage cells, as is often the case with scales on the flowering axes of holosaprophytes.

Structure of the Absorbing Organs.

On a relatively main axis there are borne tufts of smaller branches, which are lateral absorbing organs, and young buds

of inflorescence-axes. The mode of growth is represented by Fig. 1. The axis grows by means of an apical growing-point, and on it arise, in acropetal succession, similar lateral branches. But at the points of insertion of these secondary axes, other branches arise subsequently on the side towards the apex of the main axis: so the false appearance is produced of a number of tertiary axes in the axils of the secondary. Of these tertiary axes one at least is a bud of an inflorescence-axis. As a rule the tertiary absorbing organs grow more rapidly than the buds; consequently distinct tufts of small absorbing organs are visible at regular intervals on the main axis. The main axis decays from behind, and it seems probable that its lateral absorbing organs thus become separate individuals.

Taking sections through a mature but not too old portion of a vegetative axis, the following structure is revealed (Figs. 2, 3, 4, 5 a, 5 b).

(1) *Sheath*.—There is no definite regular layer of cells clothing the surface. Some of the external cells are elongated at right angles to the surface and form short *hair-like outgrowths*. But often a delusive appearance of hair-production is caused by the cells, which are elongated longitudinally, partially separating from the subjacent cells and the free portion protruding outwards. A very feeble cuticle coats the outer walls of the superficial cells. Within succeed about 2–3 layers of cells with small intercellular spaces. They are elongated longitudinally. These and the cells exposed to the surface agree in being parenchymatous cells with thin cellulose-walls, having a delicate protoplasmic lining and a large amount of cell-sap, but no starch; in fact they might be described as constituting an aqueous tissue. They may be collectively referred to under the name of the *sheath*. Amongst the sheath-cells are raphide-mucilage cells. The most striking feature about this tissue is that *straight fungal hyphae* (mycorrhizal) *run through some of the cells*. These hyphae traverse the centres of the cells running in a longitudinal direction with wonderful straightness. Here and there they

send off straight branches which run obliquely outwards or obliquely inwards (Fig. 2). There are in addition minute, blunt, nearly solid protuberances on the sides of the hyphae: these are arrested branches. The hyphae are mostly unseptate inside the sheath, but some of the older hyphae are divided by transverse walls. They are continuous with septate hyphae outside the plants and also with the hyphae occupying the deeper layers of the organ.

(2) *Exocortex*.—Within the innermost layer of sheath-cells a sudden and remarkable change in the tissue takes place. There succeeds a single unbroken and very regular layer of cells, with no intercellular spaces and having a quite different shape and strikingly different contents. These cells vary in form from narrow palisade-like cells elongated radially, to more or less square cells: but in any case they are much broader radially than the sheath-cells. They have thin cellulose-walls. But in their contents they form the greatest contrast to the sheath. *Every cell is almost filled with a coiled mycelium consisting of swollen irregular moniliform mycorhizal hyphae with densely staining protoplasm.* The protoplasm of the host-cell coats these hyphae and lines the wall; there is a conspicuous nucleus, and a relatively small amount of cell-sap. I pointed out that in the absorbing axes (root or stems) of holosaprophytic Orchids some of the outer tissue entertains living coiled mycorhizal mycelia, and that this tissue is sometimes differentiated even in the growing-point before the hyphae have reached it: to it I gave tentatively the name *exocortex*¹, and the same term may be employed to describe this single layer in the absorbing organ of *Thismia*. Very rarely one of the cells of this layer in *Thismia* is changed into a raphide-mucilage cell. No starch is found in the mature cells.

(3) *Limiting Layer*.—Within the exocortex a layer succeeds which again sharply contrasts with it. The cells are parenchymatous, radially flattened, smaller than the preceding,

¹ Percy Groom, Contributions to the Knowledge of Monocotyledonous Saprophytes, Journ., Linn. Soc. read Dec. 20, 1894.

slightly elongated longitudinally; they include no intercellular spaces. They have thin walls of cellulose, and protoplasm lining the walls and coating the hyphae. The latter are widely different from those of the preceding layer; they consist of *very slender hyphae, often spirally twisted, which suddenly swell out into conspicuous intercalary bladder-like bodies often filled with densely staining protoplasm*. Usually there is only one, or at most two bladders, in one cell. The hyphae may be traced from the exocortex and from the deeper layers of cortex into this limiting layer.

(4) *Mediocortex*.—The next two layers of cells lying within are much larger, and are more or less isodiametral and hexagonal, or the inner slightly elongated longitudinally. The intercellular spaces are small. They contain *conspicuous dead yellow mycelial masses, consisting of portions of defunct hyphae which are connected by slender portions of hyphae with one another*. In addition these cells may contain large quantities of starch. Here it may be mentioned that the starch throughout the plant assumes a red colour with iodine, as is often the case with starch in holosaprophytes. In this region of the cortex there are a considerable number of large raphide-mucilage sacs. In accordance with the terminology before suggested, these two layers constitute the *mediocortex*, characterized by containing inert refractive fungal masses. In parts of the axis these layers are immediately succeeded by the endodermis; in other thicker portions there is a third layer of parenchyma with cellulose-walls; but in this third layer the cells are markedly elongated longitudinally, and as a rule they are utterly devoid of hyphae though often rich in starch.

(5) *Endodermis* consists of a somewhat irregular single layer of feebly protoplasmic cells with thin suberised walls displaying the radial dots. The cells are elongated in a longitudinal direction, but they are considerably smaller than the cortical cells outside them.

Central Cylinder.—The stele is very narrow in comparison with the well-developed layers of parenchyma constituting

the sheath and cortex. It commences with a single tolerably regular and typical *pericycle*, the cells of which are elongated longitudinally and are narrow.

Phloëm.—There are about 6–8 minute bundles of phloëm lying against the pericycle. Each group consists of 1–3 extremely delicate sieve-tubes with terminal plates, and 1–3 slightly wider protoplasmic cells representing companion cells (in transverse section).

The different bundles of phloëm are separated by parenchymatous conjunctive tissue, which also bounds them on their inner sides.

The *xylem* is reduced to a single axial strand of very narrow annular and spiral vessels, and slightly broader, more central, reticulate-scalariform tracheae. In transverse section the strand may exhibit as few as three vessels, but never more than eight.

The structure of the growing-point of this organ at once reveals the fact that the vegetative axis of *Thismia* is absolutely unique.

The *sheath* is continuous over the apex, and the actual tip of the organ ends in a collection of sheath-cells protruding as conspicuous hairs¹. The meristem lies internally (Fig. 4), and though it is impossible to certainly distinguish the initial cells sufficiently clearly to conclude that there are three distinct histogens, still one can recognize that in the apical meristem three distinct tissues are differentiated very early—the *sheath*, the tissue extending from and including the exocortex to the endodermis, and the central cylinder. In fact the apex suggests that of an ordinary succulent monocotyledonous root, in which the absolute distinction into calyptragen, periblem, and plerome is not obvious at the extreme merismatic limit. Thus the *vegetative axis is possibly a root in which the root-cap is preserved throughout life as a living and absorbing layer* which further acts as an aqueous tissue and entertains the symbiotic fungus. Some of its cells do, however, peel off and die, but the majority persist. If this interpretation

¹ Compare a haustorium.

be correct, then the *exocortex*, inasmuch as it is the outermost layer which soonest ceases undergoing periclinal divisions and becomes earliest sharply marked off from the cells produced by the calyptragen, *is the original piliferous layer* (epiblema). Undoubtedly the cells which produce it also give rise to the layers as far as the endodermis, but it was impossible to see whether the initials of this layer and the cortex are absolutely distinct from those forming the sheath. The *limiting layer* then *corresponds with the exodermis*. The rest of the cortex is equivalent to the remaining cortical layers of an ordinary root. The section figured suggests that the plerome has initials of its own (Fig. 4). The structure of the central cylinder favours the view that this vegetative axis is a root. There is in other saprophytic roots, and in Burmanniaceae in particular, a tendency for the xylem-bundles to be developed towards the centre of the cylinder; and there is the further fact that the spiral and annular vessels tend to lie outside the scalariform-reticulate tracheides.

But another interpretation is possible, namely, that the vegetative axis is a rhizome in which the leaves are congenitally fused with the stem. This view may be illustrated by some examples which offer stages towards this process of fusion. On the tuberous rhizome of *Epipogum nutans*, the leaves are well developed but without vascular bundles, and their inner (upper) faces are so closely adherent to the axis that they have in sections to be separated by chemical means¹. In addition the lower (outer) surface of the leaves is provided with numerous absorbing hairs. If we imagine this state in the ancestor of *Thismia*, and that gradually the leaves have become congenitally fused with the axis, the present structure would result. In this case the sheath represents the leaf-tissue, the exocortex represents the epidermis of the axis, the tissue within corresponds to the cortex and stele of the stem. This view is supported by the fact stated by Johow², that in

¹ Percy Groom, loc. cit.

² Johow, Die Chlorophyllfreien Humuspflanzen, &c. Prings. Jahrb. xx, p. 475.

Dictyostega orobanchoides (Burmanniaceae) the leaves of the rhizome do produce absorbing hairs and form the absorptive organs of the plant.

There is a third possibility, namely, that the vegetative axis is a rhizome in which the leaves have been completely suppressed. I have already shown that in a hemisaprophytic Orchid (*Corysanthes*) there is an atrophy in the leaves of the absorbing rhizome. In a later paper I shall show that the same atrophy has taken place in the green Burmanniaceae to such an extent that the leaves are mere microscopical scales (cp. *Psilotum*). Further, the structure of the stele in absorbing rhizome-axes of hemi- and holo-saprophytes is frequently remarkably like that of a root (*Corysanthes*, *Burmannia*, *Corallorhiza*), so the root-like nature of the stele of the absorbing organ is no proof of its root-nature. Nor is the position of the spiral and annular vessels absolute testimony that it is a root, for even in the inflorescence-axis of *Thismia* the protoxylem is not accurately radially within the phloem and later wood; and in indubitably absorbing stems of saprophytes, the protoxylem often does not stand radially within the later formed xylem.

The fact that in some other Burmanniaceae the rhizome develops as an absorbing organ, is not of the slightest value as evidence that the absorbing vegetative axis of *Thismia* is really a rhizome. For amongst the Orchidaceae in some forms the root-system is entirely absent and the rhizome greatly developed as an absorbing and vegetative organ (*Epipogon*, *Corallorhiza*), whereas in other Orchids the roots are greatly developed and act as absorbing organs (*Galeola javanica*, *Neottia*, &c.).

But the structure of the growing-point, taken together with that of the stele, seems to me to point rather to the absorbing organ being a root, and not, as hitherto assumed, a rhizome¹. However, it seems safer to call it a thallus.

¹ See Engler, Burmanniaceae, in Engler & Prantl's Pflanzenfamilien.

Origin of Lateral Members on the Vegetative Axis.

I did not succeed in finding any sufficiently early stages of the lateral absorbing organs, but their structure and attachment to the main axis suggest that they arise endogenously in that they arise from tissue lying deeper than the sheath. The scapes develop from cells which lie deeper than the sheath (Fig. 3). Beyond making this statement I can say nothing. The very young scape could be identified as such by the feeble lobes representing the primordia of the scales. Even when the scales were clearly differentiated the young buds could be seen covered with layers of cells belonging to some external tissues.

Development of the Mycorrhizal Mycelium, and the Relations between it and the Nuclei of the Infected Cells of Thismia.

In view of the all-important functions ascribed to the nucleus of a cell, the question naturally arises as to the relation between the nucleus of the host-cell and the hyphae of the symbiotic Fungus. If the host-cells and the fungal hyphae do actually work in harmony, and if the nucleus be the guiding centre of a cell, we should expect that some intimate relations would exist between the host's nuclei and the guest's hyphae, and that possibly such relations would be reflected in the histological details of the symbionts. In the thallus of *Thismia* the coarseness of the hyphae, and their long persistence as distinct filaments, render investigation of this question simple.

The mycorrhizal hyphae in the different layers of the thallus of *Thismia* may be traced to the apex as separate living hyphae which reach nearly as far as the dividing merismatic cells. *The hypha, then, does not enter a cell till the latter has finished dividing and already possesses a nucleus clothed with a distinct permanent membrane. Thus there is no evidence of any exchange of living nuclear material between guest and host.* My material was not fixed in such a manner as to enable me to assert or deny any protoplasmic continuity

between the cytoplasm of the host and that of the Fungus, but there never was any indication of such a connexion, though each hypha is externally coated from first to last by a sheath of cytoplasm.

The *differentiation of the definite layers of the thallus is not directly caused by the mycorrhizal organism*, for the distinction of the various layers (sheath, exocortex, limiting layer, rest of cortex, and stele) appears in young portions of the apex before the hyphae have reached the cells of that region.

I did not trace the mode of entrance of the hyphae into the sheath-cells, but in all the deeper infected tissue the *Fungus enters the cell as a single slender hypha which at once grows directly towards the nucleus of the host-cell* (Figs. 6, 16). That it is not the nucleus which travels towards the hypha is shown by the fact that the nucleus preserves its original central position before and for some time after the hypha has reached it.

The later career of the mycelium represented by this single hypha depends on the position of the host-cell. As the subsequent changes are most clearly traceable in the inner layers of the cortex (mediocortex) an account will be given first of the development of the mycorrhizal Fungus in this region.

Development and Fate of the Mycelia in the Mediocortex
(Figs. 6-11).

With the penetration of the embryonic hypha, the starch-grains of the cell disappear never to return, or to reappear only when the hyphae are dead or dying. When treated with Gram's method (methyl violet) and cleared with oil of cloves containing eosin, the protoplasm of the hypha stains blue, and its numerous minute nuclei become deep blue; the cytoplasm of the cell and the main body of the cell-nucleus colour pink, but the nucleolus and some large granules in the nucleus stain deep blue. *The hypha having reached the nucleus commences to form a local pear-like or oval swelling against the nucleus.* This young bladder is really intercalary,

but as the hypertrophy is particularly on the side towards the nucleus, the thin continuation of the original hypha (i.e. the apical part) appears to spring from the young bladder at a point close to the insertion of the original slender portion of the hypha. The bladder gradually assumes a spherical form. At first it is filled with a densely pink-staining mass of cytoplasm with many nuclei. But as it attains larger dimensions vacuolation sets in. The bladder is now mature, and the further changes in it are associated with its waning vitality and death. Either the natural movements of the cell's protoplasm, or movements associated with the local hypertrophy of the hypha, cause the host's nucleus to shift its position in the cell. The young slender continuation of the original hypha now bends towards the cell-nucleus, and applying itself to the latter proceeds to form a second bladder. In the meanwhile great changes are taking place in the first bladder. Its protoplasm loses its staining intensity, diminishes in amount, and at the same time there is deposited in it a homogeneous yellow substance in which are rod-like bodies, which remind one of the regular rod-like 'bacteroids' in leguminous tubercles. This deposit appears always to commence at the proximal part of the bladder, and is not a product of the metamorphosis of the cell-wall, though it lies close within the wall and sooner or later is absolutely in contact with it. This substance gradually increases in size, the walls of the bladder collapse, and finally nothing remains but a shrunken wall covering a mass of homogeneous substance coloured by a yellow oil-like liquid, and containing numerous rod-like bodies. The older thin portion of the hypha shrinks too, and the yellow substance seems to block the connexion between its lumen and the thin portions of the hypha. So it is altogether inconceivable that any considerable conduction of liquids can take place along these thin portions of the hyphae. The second bladder soon passes through the same changes as the first, and a third bladder, or even a fourth and fifth, may be formed and repeat the process. But sooner or later the hypha passes through the cell-wall and enters

an adjoining cell which lies nearer the apex or is situated nearer the centre of the root. This young hypha again repeats the same history as in the preceding cell. Hence in the cells of the mediocortex, the youngest bladder is always to be found lying against the nucleus, although the nucleus does not preserve its original central position. In an old cell in which all the bladders are dead, the main mass of the cytoplasm, as also the nucleus and its bubble-like nucleolus, stain pink, but minute granules in the nucleus still stain blue, when treated with Gram's method as already described. No hyphae enter the raphide-mucilage sacs.

The Mycelia in the Sheath-layers.

The hyphae in the sheath behave very differently. The hypha runs straight through the middle of the cell in a longitudinal direction. The nucleus of the host-cell may be often seen lying close against this hypha. The hypha undergoes no local hypertrophies; it increases evenly in volume, so that an old hypha is merely stouter than a young one, and its contents do not stain so intensely blue. The mycelium in these sheath-layers is very long-lived, so that in old parts of the thallus it is still living and protoplasmic. There is at most a feeble deposit of excreta as represented by a more or less faint yellow colouration and minute yellow granular clumps in the protoplasm of the old hyphae.

The Mycelium of the Exocortical Layer (Figs. 12-15).

The hyphae of the characteristic exocortical layer are intermediate in behaviour between those of the sheath and those of the mediocortex. The slender hypha on reaching the nucleus does not at first undergo considerable local hypertrophy; it grows rapidly and coils about in the cell, and the older parts increase at first tolerably regularly in volume. It seems probable that the youngest part of the hypha always tends to follow the host's nucleus. For in sections one often sees this youngest part close to the nucleus, and frequently fails to find a young slender hypha distant

from the nucleus unless it be leaving the cell altogether. Eventually, however, distinct localized enlargements form on the hyphae, which thus assume bloated irregular moniliform shapes. The hyphae branch but feebly in these cells. The hyphae usually pass on to younger cells of the same layer, i.e. the hyphae tend to run longitudinally, but occasionally they send branches into the cells lying within, namely, the limiting layer. The mycelia live much longer than those of the mediocortex, and in old parts they may be found with deeply staining protoplasmic contents. When they do die, which is earlier than is the case with the mycelia in the sheath, there is little or no deposit of excreta, and all that remains of them is a shrunken clump of thin hyphal walls.

The Mycelia in the Limiting Layer (Figs. 15, 16).

The mycelia in the limiting layer in every way form a transitional stage between those of the exocortex and those of the mediocortex. The hyphae curve sharply, swell irregularly into one or two bladders which are formed only in contact with the nucleus of the host-cell; the unswollen portions retain their embryonic slender calibre. The bladders are not so regularly spherical as those of the mediocortex, but often the primitive egg-like form characteristic of their early state in the mediocortex persists. Furthermore these bladders preserve their protoplasmic contents much longer than do those of the deeper cortical layers; and often in old parts these densely protoplasmic bladders at first sight look like large single spores.

Previous Records of Formation of Intercalary Bladders on Hyphae of Mycorrhiza.

The first record of intercalary hypertrophies of mycorrhizal hyphae is that of Mollberg¹, who found them in *Platanthera bifolia* and *Epipactis latifolia*. He wrote: 'Es waren intercalare und auch terminale knopfförmige, aber auch lange

¹ Mollberg. Quoted in Wahrlich's paper.

keulige Auftreibungen, die auf den ersten Blick wie beginnende Sporenbildungen aussahen. Diese Gebilde traten auch kettenförmig hinter einander auf, waren reicher an Protoplasma als die Fadentheile und besaßen grosse Vacuolen. In der Kultur wuchsen sie wieder zu gewöhnlichen Fäden aus, ohne sich zu Reproduktionsorganen auszubilden.' W. Wahrlich¹ investigated other Orchids, and the first stage he found in the cortical parenchyma of the fungal clumps was a sac-like swelling of a hypha which soon gave off numerous branches. These latter finally form a close system of fine hyphae concealing the original sac. Kühn² showed that in the mycorrhiza of Marattiaceae mycelial clumps occur with a central bladder-like sac; in fact are just like those described by Wahrlich. Kühn figures a cell from the mycorrhiza of *Angiopteris evecta* as containing a bladder, also a hypha dilating into a terminal spherical body which is rich in protoplasm and possesses a large nucleus. This last he terms a spore with a large nucleus. But I have no doubt that the nucleus (as its size indicates) was really lying outside the spherical body and was the nucleus of the host-cell, and the sphere itself was nothing else than a young bladder. So it seems likely that in *Angiopteris*, too, the bladders form first in contact with the nucleus of the host-cell.

B. PHYSIOLOGY.

Discussion of the Behaviour of the Mycorrhizal Mycelia.

The first prominent fact with reference to the mycelia of *Thismia* is that a young hypha on entering a cell grows directly towards the nucleus of the latter. There are two possible explanations of this phenomenon. It may be that the hypha grows in that direction for purely mechanical

¹ W. Wahrlich, Beitrag zur Kenntniss der Orchideenwurzelpilze. Bot. Zeit. 1886, p. 480.

² R. Kühn, Untersuchungen über die Anatomie der Marattiaceae und anderer Gefässkryptogamen. Flora, 1889, pp. 491-497.

reasons, because the currents in the cell set most vigorously towards the nucleus, and the hypha is carried with them, much as, in flowing water, a slender submerged stem has its young parts pointed down stream. The other possible explanation is that the hypha seeks the nucleus in virtue of its chemotropism, the chemotropically active substances being manufactured or accumulated in greatest quantities nearest the nucleus of the infected cell¹. This second explanation appears to be the correct one, as will be seen from the following considerations. (i) The distribution of the endotrophic mycorrhizal hyphae in a holosaprophyte suggests that we are dealing with a chemotropic phenomenon². The coiled hyphae occur in some cells, but are absent from others equally accessible. They enter into cells of absorbing organs—roots, leaves, or stems—but avoid cells of organs which are not absorbing, whatever their morphological nature. And even in the absorbing organs the hyphae are absent from some cells (raphide-mucilage cells) though present in the contiguous one. (ii) Miyoshi³ has shown that certain chemical bodies do exercise a directive influence on the hyphae of Fungi. (iii) There is evidence that absorption of plastic substances from the host-cell by the hypha takes place most vigorously near the nucleus of the cell. For in the mediocortex and limiting layer the bladders, at first full of protoplasm, form only in contact with the host's nuclei. We cannot explain this local hypertrophy of the hyphae as due to a stoppage of substances conducted inside and along the hyphae; because of the slender nature of the portions of the hyphae connecting the bladders with one another, and because of the early death and probably the complete occlusion of those portions. So we are compelled

¹ Of course it is equally conceivable that in one cell the hypha moves towards the nucleus in virtue of a repellent action of a substance which is consumed most rapidly near the nucleus. But, as will be seen later, this view would involve a number of complicated assumptions for which there is no evidence.

² Percy Groom, loc. cit.

³ M. Miyoshi, Über Chemotropismus der Pilze. Bot. Zeit. 1894.

to conclude that the bladders are formed near the nucleus of the host-cell because it is at this point that the hypha is absorbing plastic substances most vigorously, and working them up into protoplasm more rapidly than they are conducted along the hypha. There is no indication of the wall of the hypha at this region being different in nature to the wall in the narrow portions of the hypha. (iv) Again, if the chemotropism of the hypha explains its growth in the direction of the nucleus, we may hope to find the same condition in other cases in which the Fungus is indubitably absorbing from the infected cell, i. e. in parasites. Rosen¹ observed in the parasitic *Puccinia asarina* that the haustorium nearly always grew towards the nucleus of the attacked cell, and becoming closely applied to it caused deformations of the nucleus, and even pushed deep into it, driving in the nuclear membrane. Professor Marshall Ward informs me that in *Hemileia* (of the coffee-disease) 'the haustoria often apply themselves to the nuclei of the host's cells.' The growth of the hypha towards the nucleus of the infected cell in mycorhiza, then, is not any evidence that the relation between Fungus and host is friendly and symbiotic, but is rather an indication that the hypha is absorbing food from the cell in which it lies. Still the apparent directive influence of the host's nucleus has been observed in other symbiotic forms. Professor Marshall Ward tells me that in the leguminous tubercles he found the hyphae (?) growing toward the nuclei. Schlicht² states that the mycorrhizal hyphae in *Paris quadrifolia* often penetrate the nucleus. In Orchids it is easy to see that in mature cells the hyphae usually form a coiled mycelium around the nucleus, and Frank has pointed out that often the first trace of the mycelium is to be seen near the nucleus of the infected cell.

Thus a hypha enters a cell because it is attracted thither by a chemotropically active substance, and goes towards the

¹ Rosen, Beitr. zur Kenntniss der Pflanzenzelle. Habil.-Schrift, 1892.

² Schlicht, Beitrag zur Kenntniss der Verbreitung und Bedeutung der Mycorhiza. Inaug. Diss. 1889.

nucleus because that substance is present there in the optimum proportion in that cell. Miyoshi showed that the attractive action of a solution varies with its concentration, there being an optimum strength, but the repellent action of a solution which attracted when dilute only sets in at high concentrations. The question arises as to the source of this chemotropically active substance in *Thismia*. Obviously it is not conducted along the hyphae themselves, so it must be absorbed from the cell in which the hypha lies. It must therefore be either derived from cells lying outside, or be manufactured in the cell in which the hypha actually lies. I shall endeavour to show that the latter is the case. In the first place, if the substance came from cells lying exterior to the mediocortex or even exterior to the exocortex, we can conceive of no reason why the hyphae should penetrate these deeper tissues at all. On the other hand, we should expect to find the cells outside would be most richly supplied with hyphae and the deeper cells successively poorer in hyphae. The reverse is actually the case; every cell (excepting raphide-mucilage cells) from the exocortex to the inmost limit of the mediocortex contains hyphae, whereas comparatively few cells of the sheath have hyphae. Thus the chemotropic action of the *cells* is weakest in the sheath and strongest in the cortex. In the second place, inside a single cell the chemotropism towards the *nucleus*, as judged by the curvature of the hyphae towards the nucleus, is weakest in the sheath, and strongest in the mediocortex, whilst the exocortex forms a layer transitional in this respect. In the third place, it would be apparently contrary to the laws of osmosis that a liquid absorbed from outside a cell should accumulate in greatest concentration in the neighbourhood of the nucleus. But this is not a conclusive argument, because in plants we often find liquids distributed in a manner apparently in opposition to physical laws (e.g. sugar in nerve-parenchyma of leaves).

Thus we are driven to conclude that the *chemotropically active substance attracts the hyphae, and is manufactured in the cell infected, and particularly in the vicinity of the nucleus of*

that cell ; and that this substance is constructed most feebly in the sheath, and most vigorously in the mediocortex.

But the influence of the nucleus of the host-cell is marked in another direction. Near it, in the mediocortex and exocortex, the bladders with their rich proteid contents form ; that is, there is abnormal thickening of the hyphae. Miyoshi, in the paper already quoted, shows that increasing the concentration of the nutritive liquid (which also acted chemotropically) causes a corresponding increase in the thickness of the hyphae. This at once rouses the obvious suggestion that in the mediocortex the protoplasmic bladders form near the nucleus of the cell, because it is at that point that the essential nutritive solution is most concentrated. Comparing the cells of the mediocortex and of the sheath, the effect of the position of the nucleus on the thickening of the hypha is greatest in the former and least in the latter. And in the mediocortex there is evidence that the substance absorbed from the host-cell, leading to the formation of protoplasm, is present in largest quantity in the mediocortex, and in smallest quantity in the sheath ; for the hypha in the sheath does not branch and scarcely thickens, but in the mediocortex great absorption and thickening of the hyphae take place. Hence, adopting the same line of argument as was used with reference to the chemotropically active substance, the conclusion is reached that *for their thickening and the manufacture of protoplasm the hyphae are dependent upon supplies of a nutritive substance manufactured by the infected cell, and particularly in the vicinity of the nucleus of that cell ; and that this substance is constructed most feebly in the sheath, and most vigorously in the mediocortex.* In this respect, too, the exocortex is transitional between the sheath and mediocortex in that a hypha, having entered the cell, does not at once pass out, nor does it describe sharp curves, but forms broad sweeps and slow curves round the nucleus, and in addition to undergoing general thickening (contrast mediocortex) it exhibits slight local dilatations (contrast sheath). These last are formed presumably at points which once were actually nearer the cell's nucleus, or physiologically so

in virtue of being the points at which the cytoplasmic strands connecting the hypha and nucleus attached themselves to the former.

It will be seen that the chemotropically active substance and the substance which is absorbed as food by the hyphae have the same distribution, and are both manufactured in precisely the same spots and in the same proportion. This at once suggests that the two are identical, that one substance causes both effects. However this may be, the theory here given of the behaviour of the hyphae leads to the conclusion that the cortex and the sheath are strongly contrasted as regards their metabolic processes; that in particular the cortical cells manufacture certain substances, or a substance, lacking nearly or entirely in the sheath. This contrast is indeed a matter of fact. For the young cortical cells contain a rich store of starch which only disappears when the hyphae enter, and which may reappear when the hyphae die: whilst the sheath-cells are always devoid of starch; or occasionally some of the cells of the innermost layer of the sheath possesses starch; and, precisely corresponding with this fact, it is not rare to find some of these innermost cells of the sheath possessing hyphae exactly like those of the exocortex, in which case the nuclei of these cells are larger and more deeply staining than the cells with only straight hyphae. Again, the sheath-cells are poor in protoplasm, but the cortical cells have an ordinary amount.

The behaviour of the mycelia, then, strengthens the view that *the nucleus of a cell is the centre of certain metabolic changes*; and, conversely, any observations tending to prove this action of the nucleus render the explanation above given more worthy of credence. Klebs' observations, though not confirmed as of general significance by Palla and Acqua, show that the manufacture of one carbohydrate—the cell-wall—is influenced by the nucleus. Further, he demonstrated that in the leaves of *Funaria hygrometrica* pieces of protoplasm devoid of a nucleus did not make starch, though they were capable of assimilating. In young cells of higher plants the chromato-

phores seem always to aggregate round the nucleus, and starch therefore frequently appears first in this circumnuclear region. So there is evidence that in plant-cells the manufacture of solid carbohydrates is influenced by the nucleus. But Demoor, by the use of chemical substances (chloroform) and by intense cold, succeeded in producing in *Spirogyra* a peculiar condition of the cell, in which the nucleus divided, but the cell-wall was not formed between the two nuclei. However this observation does not prove that the nucleus was the only active part of the protoplasm, nor does it necessarily imply, on the other hand, that we must ascribe to cytoplasmic activity any initiatory share in the formation of the cell-wall. Haberlandt showed as a matter of fact that the nucleus of plant-cells often wanders towards that part of the cell-wall at which irregular thickening is taking place; and this observation seems of more value than those in which the protoplasm was brought into a pathological condition by excision of the nucleus or use of abnormal stimuli. Animal physiologists have also conducted observations which have led them to conclude that the nucleus influences, or even controls, the metabolism of a cell in some directions (processes of secretion of calcium carbonate, mucilage, and animal cuticle¹).

To sum up: *the distribution, form, and mode of development of the mycorrhizal hyphae of Thismia are dependent on their constant chemotropic character, on the location of the cell infected, and on the metabolic processes taking place in that cell, particularly near its nucleus.*

And doubtless this generalization holds, at any rate for orchids and Marattiaceae. For in the mediocortex the mycelia only differs from those in *Thismia* in that numerous slender hyphae grow out from the primary bladder, and in the subsequent changes said to occur in these hyphae.

¹ Max Verworn, *Allgemeine Physiologie*, contains references to all the papers quoted. M. Verworn puts forward the view that the nucleus influences the metabolism of a cell in a manner analogous to a chloroplast. See his article *Die Physiologische Bedeutung des Zellkerns*, *Pflügers Archiv*, Bd. li. pp. 1-118.

In most saprophytes the hyphae of the endotrophic mycorrhiza do not enter parts of the shoot which are not absorbing. This is presumably because the chemotropically active substance, if present at all, is only extremely dilute and rapidly consumed by the host. If this view be correct we might expect in *Thismia* that any mycorrhizal hyphae entering the inflorescence-axis would assume the form of the sheath-hyphae. This is in fact the case, as was mentioned earlier in the paper. Further, in Fig. 3, it can be seen that, as the cortex of the thallus approaches continuity with the parenchyma of the primordium of the inflorescence-axis, the hyphae in the limiting layer of the former assume the characters of the exocortical hyphae: and the hyphae in the exocortex become straight. Employing a high-power objective it could be further seen that at the base of this primordium of the bud, in the cells continuous with the mediocortex, the hyphal-bladders became fewer and the hyphae more uniform. These facts, I think, dismiss the suggestion which might be made, that the different behaviour of the hyphae in the various layers of the thallus is due, not to any distinction in the metabolism of the cells, but to the circumstance that, as the hyphae penetrate the deeper tissues, they may become more irritable to chemotropic stimuli.

Physiological Significance of Endotrophic Mycorrhiza.

The precise source of the foods of guest and host in mycorrhiza has hitherto not been ascertained, neither has proof been given of any reciprocal interchange of nutritive substances between the two. The large dimensions of the hyphae in *Thismia* and their feeble branching in a cell rendered them peculiarly suited for such observations. Hence some comments on the physiological significance of mycorrhiza may be of interest, particularly as the conclusions drawn differ considerably from those taught by Frank, who has recently devoted much attention to the subject.

Frank gives two entirely different interpretations of the significance of the two forms of mycorrhiza—ectotrophic and

endotrophic. With regard to the ectotrophic mycorrhiza, he appropriates Kamienski's theory that the Fungus prepares food for the host; and in particular supposes that the Fungus assimilates, partly for the benefit of the host, humous compounds. But the endotrophic Fungus he regards as a living organism captured by the host. He says¹, 'Denn der Pilz ist hier gleichsam in den Wurzelzellen gefangen, wo er als sichere Beute zuletzt von der Pflanze aufgezehrt wird. Die Erscheinung entspricht, mutatis mutandis, genau dem Insectenfang der sogenannten insectenfressenden Pflanzen, und wir können daher die endotrophen Mycorrhizen als *Pilzfallen* und die betreffenden Pflanzen als *pilzverdauende Pflanzen* bezeichnen.' He then goes on to say that it is probable that the hyphae receive the whole of their nutriment directly from the external substratum, not from the host. His theory, or rather definite statement, is based upon observations made on roots of *Orchis*. He states that in the cortex the protoplasm of the hyphal clumps is drained out of the hyphae, and all the proteids resulting pass into the cells of the host; and that finally the hyphal clump consists solely of the walls of the hyphae. His evidence for these statements is that with increasing age the hyphal clumps gradually lose their power of staining with anilin-blue. Admitting this diminution of staining power and that it is due to a disappearance of proteid, still Frank fails to exclude the possibility that the proteid might be converted into cell-wall or into additional yellow excretion; nor does he show that the proteid in older parts might not have been conducted away along the slender portions of the hyphae to younger hyphae, as is often the case with the hyphae of any ordinary mycelium. Still it is quite possible that Frank may be correct in his assumption, though not in the dogmatic expression of it as a proven fact. But with regard to his analogy of endotrophic mycorrhiza with an insectivorous plant some remarks are called for. A captured insect derives all its food from the outside world

¹ Frank, Lehrbuch, p. 267. See literature given also.

and none from its captor; and further it is entirely consumed by the latter, nor has it a chance afforded of propagating its species after capture. Do these two conditions hold for the endotrophic Fungus?

With reference to the absorption of food, it has been shown earlier in this paper that the *hyphae absorb food manufactured by the cortical cells in the mycorrhiza of Thismia*, and that this absorption by the hyphae is accompanied by a large increase in their protoplasm. Now the hypertrophied hyphae (in the cortex, and occasionally in the inner cells of the sheath) are only found in cells which, before the hyphae entered, contained starch. When the hyphae penetrate these cells the starch disappears, and does not reappear in them till there is evidence that the hyphae cease, or almost cease, manufacturing protoplasm. In the mediocortex, as bladder after bladder forms in a cell, starch remains absent, but when the formation of new bladders stops and the hyphae all die, starch may reappear¹. These facts tend to show that the *starch disappears and remains absent because the carbohydrates are being consumed in the production of proteids*, and not because the cell ceases to be able to manufacture carbohydrates. But this does not imply that the soluble carbohydrate (sugar) is absorbed as such by the hypha. It may be that the hypha merely causes more vigorous osmotic currents to the cell which entertains it, and thus brings a larger supply of nutritive material, which with the carbohydrate forms proteid in the cell. Or it is possible that the sugar is directly absorbed by the hypha, and the proteid built up in the hypha. Both soluble carbohydrates and soluble proteids act chemotropically on fungal hyphae. But whether it be proteid or simply carbohydrate (sugar) which is manufactured near the host's nucleus and is then absorbed by the hypha, the co-operation of the cell and hypha has three effects. First, as evidenced by the increased size and staining

¹ I have noted the same fact in orchidaceous mycorrhiza, that starch appears in the old cells of the mediocortex when the hyphal clumps are dead. So Frank is incorrect in saying that starch is not present in cells containing the fungal hyphae.

powers of the nucleus of the cell¹, there is an *increase in the metabolic activity of that cell*. Secondly, there is a sudden *rise in the assimilation of nitrogen which exhibits itself in the rapid synthesis of proteids*. Thirdly, the *carbohydrate manufactured by the host is concerned in the formation of proteid in the mycelium*.

After a time the protoplasm of the hypertrophied bladder in the mediocortex diminishes in amount; and, *pari passu*, there is a deposition in the bladder of a yellow mass with rod-like bodies. This yellow mass with the bodies is absolutely useless to the host; for, as the mycorrhiza decays, these masses remain unaltered though the protoplasm of the dying host-cells is conducted away, and at the exposed and disintegrated surface of the decayed mediocortex the yellow masses may be recognized. Hence the *protoplasm of the hyphae in the mediocortex is partially broken down into resistant excreta absolutely useless to Thismia*. As the protoplasm of the bladder is vanishing and the yellow mass appearing, the bladder shrinks and its wall collapses. A liquid therefore leaves the bladder. Now evidence has been given earlier in this paper that the liquid is not conducted away along the slender portions of the hyphae, because they die soon and are occluded, and because they always are slender in calibre. Hence it must be concluded that the *moribund mycelium pours a liquid into the host-cell*. As to the nature of this liquid one cannot do more than guess.

Thus it is established that in the mediocortex of *Thismia* there is an interchange of material between Fungus and host, and that that material is manufactured by the two symbionts respectively. In the Orchidaceae, in *Paris*, Marattiaceae, &c., doubtless there is likewise an absorption on the part of the hyphae of substance manufactured by the host; for there are indications of the effect of the nucleus of the cell on the

¹ Compare the researches of animal physiologists in showing that the size and staining power of a nucleus is a good index of the metabolic activity of that cell (Heidenhain on secretory cells, Hodge and G. Mann on nerve-cells).

growth of the hyphae, as there are indubitable proofs of the influence of the hyphae in leading to an increase in the size and staining powers of the nuclei. So there is no reason to doubt that the symbiosis in the mediocortex of these plants has the same significance as in *Thismia*.

To sum up, then. *Although the hyphae of endotrophic mycorrhiza in the mediocortex die soon, the root (or rhizome) cannot be said to act like the digestive organ of an insectivorous plant, because the protoplasm of the hyphae is manufactured partly at the expense and through the agency of the host; and, further, there is, at present, no direct evidence that the hypha renders its proteid contents to the host.*

The second point with reference to Frank's analogy may be now considered. Is the organism (Fungus) completely killed by the host (root or rhizome)? The evidence all seems to point in the other direction. In all the Orchidaceae and Burmanniaceae I have examined, in all the cases of orchidaceous saprophytes investigated by others, and in endotrophic mycorrhiza generally, it appears that there are in the outer layers of the absorbing organ hyphae which live as long as the organ itself. They are many of them continuous with hyphae entirely outside the host, and extending out into the substratum. The number and development of these hyphae extending outside the plant varies much in different plants, often being inconsiderable (*Thismia* and some Orchids). It seems probable, therefore, that Frank is correct in regarding these free hyphae as not of supreme importance in supplying nutriment to the mycorrhiza, though they often ramify closely over dead vegetable fragments (*Thismia*). In *Thismia*, in particular, the whole surface of the thallus appears capable of absorbing liquids; and here also the existence of a sheath renders possible the observation that *frequently the free hyphae are deserting the host, not entering it, a fact which seems to give the key to the puzzle.* In all the cases of saprophytes with endotrophic mycorrhiza which I have observed, the free external hyphae can be traced continuously from the substratum into cells capable of manufacturing

starch. In *Thismia* they continue from the exterior into the exocortex with undiminished general calibre. From this layer no plastic material can be conducted along and inside the hyphae to the dead mediocortical hyphae. On the other hand, food can easily be transported along the wide hyphae towards the exterior. Plastic material is undoubtedly being continuously absorbed from the host by the exocortical hyphae, for starch does not appear in the infected cells (though it frequently is found in the limiting layer cells, in which the bladders still have their dense protoplasmic contents). Hence it is safe to assume that *the exocortical hyphae act as haustoria for the benefit of the hyphae lying in cells outside them*. These external hyphae, then, derive from the host a considerable amount of nutriment, which enables further growth into the substratum outside the host. This method of regarding the significance of the hyphae of the sheath and exocortex is strongly supported by additional facts referring to *Thismia*. As was before stated, the old end of a thallus gradually decays. As the parts die the cells lose their turgidity and collapse, so that the whole organ, in that region, shrivels to a mere thread. In such disintegrating parts proteid contents may be seen in the exocortical hyphae, even after the death of the cells which entertain them, and subsequent to the complete disappearance of the proteid contents of those cells. But these hyphae eventually die and their walls become thick and glistening. The hyphae of the sheath die still later, and in dead parts of the thallus living hyphae may be traced continuously from them to the outside of the plant. In these dying and dead parts I found numerous cells, particularly superficial cells, crowded with fungal spores produced by hyphae which could be traced into the deeper layers of the sheath, and also were continuous with hyphae ramifying over the surface of the organ. These hyphae were indistinguishable from the normal mycorrhizal hyphae, excepting that they did not run straight in a longitudinal or longitudinal-oblique direction in the sheath. In spite of the fact that my sections showed the two sets of hyphae coming close together, I could

not actually establish their continuity. Hence I cannot positively assert that the spore-producing hyphae belonged to the mycorrhizal Fungus, yet there seems little doubt that such was the case. If the sporogenous hyphae were really a part of the mycorrhizal mycelium, their irregular direction of growth must be attributed to the removal of the directive influence of the host-cells and to the breaking down of the protoplasm of the latter. If, on the other hand, the sporogenous hyphae belong to some other Fungus which could not enter the thallus as a parasite till the latter had already lost some of its vitality and the mycorrhizal hyphae were already weakened, then the obvious analogy between the symbiosis of mycorrhiza of *Thismia* and phagocytosis becomes still more marked. In *Galeola javanica* I further saw some of the superficial cells packed with similar spores. Wahrlich observed and figured spores, just like those in *Thismia*, in the velamen of Orchids infected with mycorrhizal hyphae; and further was able to prove that they were produced by segmentation of the hyphae and rounding off of the cells (i. e. this represented the oïdium-stage of life-history), as I also believe was the case in *Thismia*. But Wahrlich makes no definite statement that he actually observed the continuity between the mycorrhizal hyphae and these spore-producing hyphae, though one reading his paper would gather that he had seen such a connexion. Mollberg also saw similar spores in the mycorrhiza of *Platanthera* and *Epipactis*. Kühn detected, inside Marattiaceous mycorrhiza, some thick-walled spores. Schacht, Reissek, and Wahrlich obtained *Fusisporium*-spores from cultures of orchid-mycorrhiza; and I saw these *Fusisporium*-spores on every thallus of *Thismia*. However, apart from this suggestive, though not conclusive, evidence of spore-production by the mycorrhizal hyphae, there is quite sufficient proof that the *endotrophic Fungus is not wholly destroyed, but on the contrary there is strong reason for believing that in the outer layers of the absorbing organ it actually profits by the symbiosis.*

Hence there is no justification for giving the misleading name of fungus-trap (*Pilzfalle*) to mycorrhiza, or for referring

to the host as a fungus-digesting plant (*pilzverdauende Pflanze*).

Having dismissed the first part of Frank's view as to the biological significance of endotrophic mycorrhiza, the second question arises, 'Is there any sufficient reason for his assumption that the physiological meaning of the endotrophic is different from that of the ectotrophic mycorrhiza?' Three series of facts all point in the same direction in answer. First, all known holosaprophytes (with one exception?) possess mycorrhiza; but it may be either ectotrophic (*Monotropa*, &c.), or endotrophic (Orchids, &c.). Secondly, in Ericaceae, Epacridaceae, &c., though the mycorrhiza is endotrophic, the hyphae do not penetrate deeper than the superficial cells of the root. This type may be regarded as affording a transition-stage between the complete endotrophism of Orchids and *Thismia*, and the ectotrophic condition of *Monotropa*, forest-trees, &c. Thirdly, in endotrophic mycorrhiza it is often the case that many hyphae radiate out into the substratum; on the other hand, in the ectotrophic mycorrhiza, hyphae frequently penetrate the superficial cells of the host's root (at any rate in older parts of the root); and in the ectotrophic mycorrhiza of *Pinus* roots, which is occasioned by *Polysaccum*¹, some of the hyphae actually dip deep into the tissue of the root, at the same time absorbing so vigorously as to play havoc with the infected tissues. Thus in ectotrophic mycorrhiza the hyphae are not always epiphytic, nor in endotrophic mycorrhiza are they exclusively endophytic.

Hence *the distribution of the two forms of mycorrhiza and the occurrence of transition-stages between their extreme forms militate against the view that the physiological significance is not the same in both*. They both probably work in one general constant manner. Mycorrhiza is, then, either a highly adapted and symbiotic community beneficial to both symbionts, or it is a pure matter of infection of a plant by a Fungus, and there is a constant struggle between host and the would-be parasite.

¹ E. Bruns, Beitrag zur Kenntniss der Gattung *Polysaccum*. Flora, 1894, pp. 67-75.

At present the weight of evidence seems to lie on the side of the symbiotic view. In favour of it are Frank's rough observations on forest-trees deprived of the Fungus, and the facts given with reference to *Thismia* showing that there is a mutual interchange of material. The distribution of the hyphae, however, might as readily be explained on the infection-view as on the symbiotic theory, now that it has been shown, at any rate in endotrophic mycorrhiza, to be as largely a matter of chemotropism.

Viewing the mycorrhiza of *Thismia* as a case of symbiosis between host and Fungus, it will be seen that there is one point of strong similarity to the symbiosis between low Algae and nitrogen-fixing Bacteria, and to the relation between the Fungus of leguminous tubercles and the host, namely, that there is an increased assimilation of nitrogen and manufacture of proteids. In his beautiful piece of work on a nitrogen-fixing Bacterium, Winogradski showed that the amount of nitrogen fixed was proportional to the amount of dextrose supplied. Transferring this idea to the case in which Bacteria and Algae together fix free nitrogen, it seems fair to conclude that the Alga supplies the carbohydrate. Again, leguminous plants may be cultivated in ordinary water-cultures and tubercles develop. Here the carbohydrate is indubitably supplied by the host, and Professor Vines' experiments showing that adequate illumination was essential to ensure the development of tubercles are thus explicable¹. With regard to ectotrophic mycorrhiza on green plants, there is no evidence that the Fungus absorbs carbohydrates from the host, though there is the collateral evidence in green Orchids that in endotrophic mycorrhiza carbohydrates are absorbed (cp. remarks on *Thismia*). It is easy to comprehend the advantage of this arrangement accruing to a mycorrhizal Fungus on a green plant which can assimilate carbon dioxide. But does the

¹ Professor Vines informs me that he has found that when Beans are germinated and grown in the dark, tubercles are developed on the roots of the seedlings: it would appear that, in this case, the necessary carbohydrate is supplied from the reserves in the cotyledons.

same hold for hosts which have no chlorophyll? Now Acton¹ has shown that certain carbohydrates and bodies like carbohydrates, including extract of humus, can be absorbed by roots of ordinary green flowering-plants and assimilated. Combining Acton's results with those of Boehm and Meyer, we assume that this assimilation is absolutely independent of light or chlorophyll. So it is *a priori* probable that flowering-plants devoid of chlorophyll can also utilize such organic compounds to supply themselves with carbohydrates, and in particular to build up starch. In this paper I show that the Fungus in the holosaprophytic *Thismia*, in fact, does derive its carbohydrate, largely and possibly entirely, from its host. Altogether there is much to indicate the probability that in all cases of symbiosis between a Fungus (including Bacteria) and another plant, the Fungus receives carbohydrates from its symbiont.

Again, we know that Fungi (including Bacteria) can readily utilize unoxidized or feebly oxidized compounds of nitrogen (NH_3 , HNO_2) and sulphur (H_2SO_3 , $\text{H}_2\text{S}_2\text{O}_3$), which are less easily, or not at all, assimilable by green plants; and on the other hand that Fungi wholly or partially lack the power of employing nitrates, or to less degree sulphates, as food. In the cases of symbiosis of Bacteria and Algae, and in the leguminous tubercles, there is, despite of Frank's researches, sufficient reason for the belief that the fixation of free nitrogen does not take place in the absence of the Bacterium or Fungus. This fact, together with the sudden increase of assimilation of nitrogen as shown in *Thismia*, suggests that in symbiosis the Fungus always absorbs, for the benefit of the community, unoxidized or feebly oxidized nitrogenous bodies which are not easily worked up by the host. This would explain the utility of mycorrhiza to plants growing in the shade of the forest or devoid of chlorophyll, and with their absorbing organs dipping in humus. Fresh humous bodies are characterized by their poverty in nitrogen of any sort; and what is

¹ Acton, The Assimilation of Carbon by Green Plants from certain Organic Compounds: Proc. Roy. Soc., vol. xlv, p. 118, 1889.

present is in the form of ammonia and feebly oxidized organic compounds. And, again, Schimper's¹ observations show that the reduction of nitrates, sulphates, and possibly phosphates, by higher plants is dependent on the co-operation of light and chlorophyll. Further, the suggestion that the Fungus is supplied direct from the substratum with its nitrogen in the form of feebly oxidized nitrogenous bodies finds support, I think, from Professor Vines'² observations showing that the development of tubercles in the Leguminosae is inversely proportional to the amount of nitrate supplied to the plants. Here the nitrate is easily assimilated by the well-lighted leguminous plant itself, and is rapidly conducted away from the roots, whereas it is probable that the Fungus, like a typical Fungus, does not readily accept nitrates as food³.

But I hope soon to publish some experimental results confirming or disproving these hypotheses.

In conclusion I desire to express my thanks to Mr. H. N. Ridley for the material used in this investigation, and for the coloured illustration of the whole plant, which was drawn by the Singapore artist De Alwis; also to Professor Vines for the continued hospitality which permitted the carrying out of this research in the Botanical Laboratory of the University of Oxford.

¹ A. F. W. Schimper, Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. *Flora*, 1890, p. 207.

² S. H. Vines, On the Relation between the Formation of Tubercles on the Roots of Leguminosae and the Presence of Nitrogen in the Soil. *Annals of Botany*, 1888, p. 386.

³ Lawes & Gilbert suggest, too, that ectotrophic mycorrhizal hyphae enable the plant infected to acquire nitrogen otherwise not easily assimilated by the plant.

EXPLANATION OF FIGURES IN PLATES XIII AND XIV.

Illustrating Mr. Groom's paper on *Thismia Aseroe* (Beccari) and its *Mycorrhiza*.

Abbreviations.—*ec*=exocortex. *enc*=endocortex. *end.*=endodermis. *ll.*=limiting layer. *mc*=mediocortex. *pc*=pericycle. *ph*=phloëm. *ph.s*=sieve-tubes.

Figs. 1-16. Illustrations of the structure of the thallus, or absorbing organ.

Fig. 1. Showing base of inflorescence-axis (*f.a.*) $\times 4$.

Fig. 2. Longit. section rendered slightly diagrammatic. (Zeiss A. Oc. 4, then reduced to $\frac{3}{8}$.)

Fig. 3. Trans. sect. showing endogenous origin of inflorescence-axis rendered slightly diagrammatic. (Zeiss Apo. 16 mm. Comp. Oc. 4.)

Fig. 4. Longit. median section of growing-point. (Zeiss Apo. 8 mm. Comp. Oc. 8, then reduced to $\frac{3}{8}$.)

Fig. 5 *a*. Trans. sect. of central cylinder. (Zeiss D. 3, then reduced to $\frac{3}{8}$.)

Fig. 5 *b*. Trans. sect. of portion of 5 *a*. (Zeiss D. 3.)

Figs. 6-11. Successive stages of development of hyphae in cells of mediocortex : longit. sect. (Fig. 9. Zeiss D. 3, all the rest Zeiss $\frac{1}{2}$ oil. Oc. 2.)

Fig. 12. Cell of exocortex before entrance of hypha : longit. sect. (Zeiss $\frac{1}{2}$ oil. Oc. 2.)

Fig. 13. Ditto shortly after penetration of hypha. (Magn. ditto.)

Fig. 14. Longit. sect. of 2 cells of exocortex representing the third and fourth stages of development of the hyphae. (Zeiss $\frac{1}{2}$ oil. Oc. 2.)

Fig. 15. Longit. sect. showing a cell of exocortex with hyphae not far from maturity, and two cells of the limiting layer representing the third and fourth stages of the hyphae. (Zeiss $\frac{1}{2}$ oil. Oc. 2.)

Fig. 16. Longit. sect. showing the first and second stages of development of the hyphae in two cells of the limiting layer. (Magnification ?)

Fig. 17. Trans. sect. of the central cylinder of the inflorescence-axis. (Zeiss D. 3, and subsequently reduced to $\frac{3}{8}$.)

Fig. 18. Trans. sect. of part of staminal tube, showing lobes forming an arch over a nectary.

Fig. 19. Whole plant, showing its habit and the substratum of decaying leaves. (Nat. size. Drawn by De Alwis.)

Fig. 20. View of the staminal tube from the outside after the removal of the perianth-tube. (Magnified. Drawn by Mr. A. H. Church.)

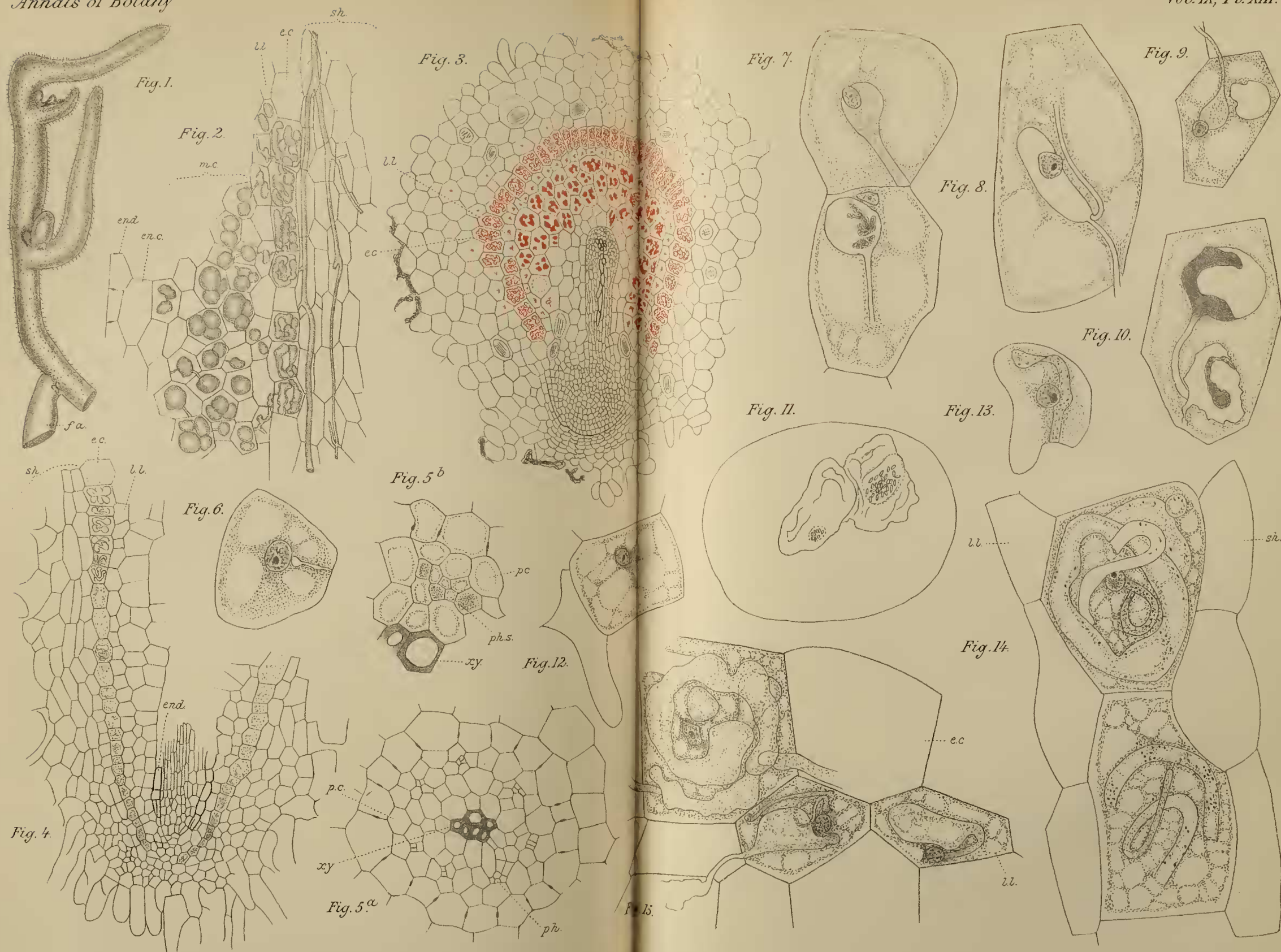


Fig. 17.

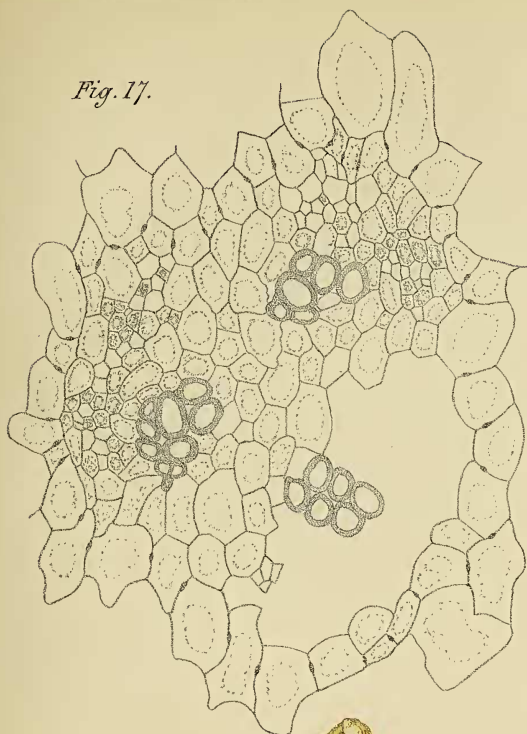


Fig. 18.

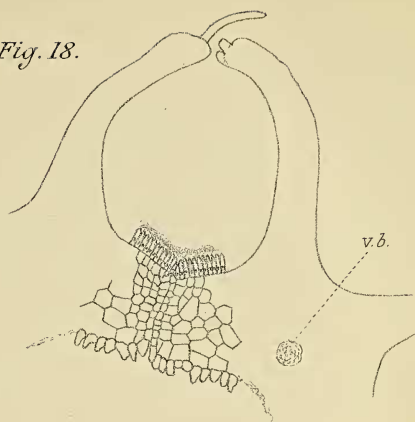


Fig. 16.

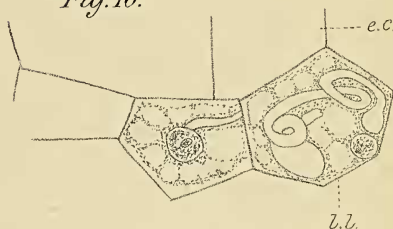


Fig. 19.



Fig. 20.

